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(54) Title: **METHODS FOR PRODUCING RECOMBINANT PROTEINS**

(57) Abstract: Methods are provided for producing and recovering recombinant proteins from plant tissue. The methods of the invention find use in the commercial processing of grains, particularly in the wet-milling of corn kernels. The methods involve steeping plant tissue and recovering the recombinant proteins in steep water. The methods additionally involve optimizing nucleic acid constructs and plants for the recovery of recombinant proteins from plant tissue.

## METHODS FOR PRODUCING RECOMBINANT PROTEINS

## FIELD OF THE INVENTION

The invention relates to the field of biotechnology, particularly to the production of recombinant proteins in plants. The invention further relates to methods for recovering the recombinant proteins from transgenic plants.

## 5 BACKGROUND OF THE INVENTION

One of the fundamental achievements of the field of the genetic engineering is the ability to genetically manipulate an organism to produce a protein that the organism was not capable of making prior to human intervention. Typically, the production of such a protein is brought about by facilitating the insertion of a  
10 recombinant DNA molecule into an organism. Nucleotide sequences within the recombinant DNA molecule contain the necessary genetic information to direct the host organism to produce the desired recombinant protein. Using such an approach, genetic engineers have modified a variety of eukaryotic and prokaryotic organisms, including bacteria, fungi, animals, and plants, to produce a wide array of recombinant  
15 proteins.

Recombinant proteins have had a major impact on agriculture, particularly on crop plants. Recombinant proteins have been used to provide new traits to crop plants which improve their performance in the field. Transgenic corn and cotton plants that have been genetically engineered to produce a bacterially derived insecticidal protein  
20 are now widely utilized by farmers. Genetic engineers have also provided the agricultural community with a variety of genetically engineered crop plants that produce proteins which increase a crop plant's tolerance to certain herbicides. Such genetically engineered, herbicide-tolerant soybeans, corn, cotton, and canola are now routinely used in agriculture.

25 While genetic engineers have achieved resounding successes with the development of such insect-resistant and herbicide-tolerant crop plants, they have not yet reached a similar level of achievement in their attempts to use plants, particularly crop plants, as synthesizers of recombinant proteins for uses such as therapeutic

























































All publications and patent applications mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and  
5 individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the  
10 appended claims.















